REMARKS

The Examiner has objected to the claims, specification, and figures because they contain sequences that are not identified by SEQ ID NO. This amendment supplies SEQ ID NOs to the claims, specification, and the Figures.

Pursuant to the requirements of 37 C.F.R. §§ 1.821-1.825, Applicants submit the enclosed Sequence Listing and computer readable form (CRF). The nucleotide and amino acid sequences disclosed in the specification and drawings may be found in computer readable form in file 990278.txt on the enclosed diskette and are presented in the paper copy of the Sequence Listing, enclosed.

Applicants hereby certify that the Sequence Listing in computer readable form supplied on the enclosed diskette as file 990278.txt is the same as the substitute copy of the Sequence Listing attached hereto. The material presented in computer readable form is not new matter because it presents sequences the same as those disclosed in the specification, as filed.

Applicants believe that the requirements of 37 C.F.R. §§ 1.821-1.825 have been met.

Respectfully submitted,

WEBB ZIESENHEIM LOGSDON
ORKIN & HANSON, P.C.

By _____
Barbara E. Johnson
Attorney for Applicants
Registration No. 31,198
700 Koppers Building
436 Seventh Avenue
Pittsburgh, PA 15219-1818
Telephone: 412-471-8815
Facsimile: 412-471-4094

MARKED-UP AMENDED SPECIFICATION PARAGRAPHS

Page 13, first complete paragraph (beginning on line 19)

Extraction of genomic DNA and the Southern analyses were performed as described previously (Schoenmakers et al., 1994). DNA from YACs, cosmids, PCR products and oligonucleotides was labelled using a variety of techniques. For FISH, cosmid clones or inter-Alu PCR products of YACs were biotinylated with biotin-11-dUTP (Boehringer) by nick translation. YAC DNA (100 ng) was amplified by inter Alu PCR (P1: CTGCACTCCAGCCTGGG (SEQ ID NO: 1), P2: TCCCAAAGTGCTGGGATTACAG (SEQ ID NO: 2). After initial denaturation for 5 min at 94°C, 30 amplification cycles were performed each consisting of denaturation for 1 min at 94°C, annealing at 30 sec at 37°C and extension for 6 min at 72°C, and with a final extension at 72°C for 10 min. Amplified DNA was purified with QIA Quick PCR Purification kit (Qiagen). For filter hybridizations, probes were radio-labelled with alpha-^{32}P-dCTP using random hexamers (Feinberg and Vogelstein, 1984). In case of PCR-products smaller than 200 bp in size, a similar protocol was applied, but specific oligonucleotides were used to prime labelling reactions. Oligonucleotides were labelled using gamma-^{32}P-ATP.

Page 28-32, the table

TABLE 3: 8q12 STS primer sequences, annealing temperatures and expected PCR product sizes

Amplimers on published sequences centromeric contig

STS name	Nucleotide sequece 5' – 3'	Product size (bp)	T_a (°C)
LYN	GGAAGGAAAGGAAAGGAGA (SEQ ID NO: 3) GGTTTGGGTGTTTGGTGTG (SEQ ID NO: 4)	194	60

MOS	GAGCTCAACGTAGCAAGGCT (SEQ ID NO: 5) AACTGTCCTCCAGTGCGG (SEQ ID NO: 6)	203	60
PENK	TAATAAAGGAGCCAGCTATG (SEQ ID NO: 7) ACATCTGATGTAAATGCAAGT (SEQ ID NO: 8)	75-83	58
D8S96	TCTCTACCTCGACATACTCCTGC (SEQ ID NO: 9) GTCAGAAGTGCCAATCAGACTG (SEQ ID NO: 10)	113	60
D8S108	CAAACCTTGAATTACAAAACAG (SEQ ID NO: 11) TGTTAATATTAGACCACCTTTC (SEQ ID NO: 12)	116	58
D8S125	TTCTGTTTCCTGGTCTCAGTAGC (SEQ ID NO: 13) CACATGCATATGTGTATGTGTGTG (SEQ ID NO: 14)	144	62
D8S165	ACAAGAGCACATTTAGTCAG (SEQ ID NO: 15) AGCTTCATTTTTCCCTCTAG (SEQ ID NO: 16)	138-152	58
D8S166	GATTGTGTCATTGCACTCCA (SEQ ID NO: 17) ACAAGGAAGTTCCTTTTTGG (SEQ ID NO: 18)	116	58
D8S285	GCATCACACAGAATCTTTG (SEQ ID NO: 19) ATGGGTTTATGGCCTTTAC (SEQ ID NO: 20)	108-124	60
D8S1069	AGCACAGTGGATATTTTTAGGC (SEQ ID NO: 21) GGGGCTCACACAGAAGTTAA (SEQ ID NO: 22)	221	60
D8S1516	GTCCCCCATCAACATGCTG (SEQ ID NO: 23) CTCATCTTGTTTTCATAGTGTTCC (SEQ ID NO: 24)	174	60

D8S1661	TCTCATGCATGTTTCCTGTTG (SEQ ID NO: 25) GTTGGGGTCATTAAACACTAGTCA (SEQ ID NO: 26)	126	60
D8S1816	TGCACCCTTAAAAAGCATCG (SEQ ID NO: 27) ACTTGCGAACATGGGATCAC (SEQ ID NO: 28)	143-147	60
D8S1828	AGTGCTGTTTTTACTTCTGTACG (SEQ ID NO: 29) GCAAGACTCTGTCTCAGGA (SEQ ID NO: 30)	198-238	60
AFMB055WG9	CTCCCAACCCACCCGAC (SEQ ID NO: 31) TGAAAACCATAATCTCTGATGTTGC (SEQ ID NO: 32)	218	60

Amplimers on newly isolated STSs centromeric contig

STS name	**Nucleotide sequence 5' - 3'**	**Product size (bp)**	**T_a (°C)**
CH33	CCTTTGGCTGGGGTTTATA (SEQ ID NO: 33) GGCCTATGAAGCAAGAGAG (SEQ ID NO: 34)	168	60
CH34	GTACCCAGAAGGCAAGTAA (SEQ ID NO: 35) GTGAAAAGGCAGAAATTAG (SEQ ID NO: 36)	145	60
CH37	TTGCATGAGAATGGAAATG (SEQ ID NO: 37) GGCGTTACTTTCCTTTTGT (SEQ ID NO: 38)	176	58
CH69	AGTGCTTACAATAGGGTGAG (SEQ ID NO: 39) CCATCCAGAAAGACCATAAT (SEQ ID NO: 40)	336	60
CH122	TTTGTCTTTGATTTTTATGG (SEQ ID NO: 41) TGACCAACATACTGCCTAGT (SEQ ID NO: 42)	250	57

CH129	CTGAATCCCAGAACAATATA (SEQ ID NO: 43) AGGGTAAGTATGTCCTTTAA (SEQ ID NO: 44)	110	60
CH273	ATAATGTTGAGACTTTGAGA (SEQ ID NO: 45) AAATGTTTATCCTAATTGTA (SEQ ID NO: 46)	157	58
CH274	CAGGTGAGTGGATGGTGTAA (SEQ ID NO: 47) CAAGGGGAGACCAAATCATC (SEQ ID NO: 48)	241	60
CH277	AATGGCTATGAGGTTGTTTT (SEQ ID NO: 49) CACATCCTTTCATTTTAGCA (SEQ ID NO: 50)	122	58
CH280	GGGCTGATGTTCCATTAACT (SEQ ID NO: 51) GCTTCAACACCAAAAATGCT (SEQ ID NO: 52)	163	58
EM76	CTGGGAAGAGATCAAAATTC (SEQ ID NO: 53) TAAAGAGACAGCACCACAAA (SEQ ID NO: 54)	220	60
EM156	AGTAGCAGCAGCAACAGTCA (SEQ ID NO: 55) TGCGCTATTCAGAGAAGATG (SEQ ID NO: 56)	160	60
EM216	CAGTCAGTTCCAGAGGTCATTT (SEQ ID NO: 57) TAGGGAGGGCTTTAATAGTGTT (SEQ ID NO: 58)	255	50?
END1	GCTCACTTCACTCCTACCC (SEQ ID NO: 59) CAACCAACCACTAAAAACG (SEQ ID NO: 60)	161	60
END2	GTGATTTTACAGCCATTTT (SEQ ID NO: 61) TGTAATTTTCAACCAGAAG (SEQ ID NO: 62)	91	50

TC65.2	TACAAACCGGGAGAAAACAG (SEQ ID NO: 63) TTACAGCATTTCCGATTTTG (SEQ ID NO: 64)	232	58

Amplimers on published sequences centromeric contig

STS name	**Nucleotide sequence 5' - 3'**	**Product size (bp)**	**T_a (°C)**
CYP7	TTGACTTTTAAATATGATAGGTAT (SEQ ID NO: 65) ACTTTTATTTCTGAAAGATGAATCA (SEQ ID NO: 66)	1600	55
D8S260	AGGCTTGCCAGATAAGGTTG (SEQ ID NO: 67) GCTGAAGGCTGTTCTATGGA (SEQ ID NO: 68)	187-213	60
D8S507	TTCCTCAGAGCAGTTCAAAG (SEQ ID NO: 69) TAATCTTGCCCCAGTGAGAG (SEQ ID NO: 70)	155-169	60
D8S1113	ATGCAAAGATGAACCAGGAA (SEQ ID NO: 71) CCCTGGACTCATGGTACTTG (SEQ ID NO: 72)	216	62
D8S1505	GGATTTTAAGTTTCTACAAAGGGA (SEQ ID NO: 73) ACAATTTCCATGAAGTGTTCACC (SEQ ID NO: 74)	328	58
D8S1723	AGCTCAATGGCACGTCCTTT (SEQ ID NO: 75) ACTGCTGACTCAGAGCCTGG (SEQ ID NO: 76)	235-243	60

Amplimers on newly isolated STSs telomeric contig

STS name	**Nucleotide sequence 5' - 3'**	**Product size (bp)**	**T_a (°C)**
CH31	TCACAGAATAATACAGGAT (SEQ ID NO: 77) GATCACTGATGATACTAGG (SEQ ID NO: 78)	220	58

CH32	ATTTGCCTCAGTGTTGCAG (SEQ ID NO: 79) ATTTTCAGGAGGTCAGGGA (SEQ ID NO: 80)	245	62
CH35	CAAAATGACTTATGCTGAA (SEQ ID NO: 81) TCTATACAGGGCATTGTGA (SEQ ID NO: 82)	165	60
EM73	AAAGCAAGACCCTGTAAAGC (SEQ ID NO: 83) CTTGGGCTCTATTTTGTGAA (SEQ ID NO: 84)	351	60

Page 37, second complete paragraph (beginning on line 17)

PCR amplifications were carried out essentially as described before (Schoenmakers (1994)). The following amplimers were used to generate a PLAG1 exon 1 probe (5'-CAA TGG CTG CTG GAA AGA GG-3' (SEQ ID NO: 85) and 5'-CCC GTC CGC CGC CTC TAC ACC-3' (SEQ ID NO: 87) and AGG GTC GTG TGT ATG GAG GTG A-3') (SEQ ID NO: 88), a PLAG1 3'-UTR probe (5'-aca tgg cat ttc gtc tca ct-3' (SEQ ID NO: 89) and 5'-CCA CAA TGG CTC TAG AT-3') (SEQ ID NO: 90) and a CTNNB1 exon 1 probe (5'-TGT GGC AGC AGC GTT GGC CCG GC-3' and 5'-CTC AGG GGA ACA GGC TCC TC-3') (SEQ ID NO: 92).

Page 37, third complete paragraph (beginning on line 28, and continuing on to page 38)

Rapid amplification of 3' cDNA-ends (3'-RACE) was performed using a slight modification of part of the GIBCO/BRL 3'-ET protocol. For first strand cDNA synthesis, adapter primer (AP2) AAG GAT CCG TCG ACA TC(T) 17 (SEQ ID NO: 93) was used. For both initial and secondary rounds of PCR, the universal amplification primer (UAP2) CUA CUA CUA CUA AAG GAT CCG TCG ACA TC (SEQ ID NO: 94) was usedas "reversed primer". In the first PCR round the following specific "forward primers" were used: i) 5' – CAA TGG CTG CTG GAA AGA GG-3' (SEQ ID NO: 95) (exon 1) or ii) 5' –

AGA ATT TGG GCC TCA GAC AAG ATA-3' (SEQ ID NO: 96) (3'-UTR, exon 5). In the second PCR round the following specific forward primers (nested primers as compared to those used in the first round) were used: i) 5'-CAU CAU CAU CAU GGC CGG AGG GAG GAT GTT AA-3' (SEQ ID NO: 97) (exon 1) or ii) 5'-CAU CAU CAU CAU ATT GTC CTG GGT TGA TTA TGC AT-3' (SEQ ID NO: 98) (3'-UTR, exon 5). CUA/CAU-tailing of the nested, specific primers allowed the use of the directional CloneAmp cloning system (GIBCO/BRL).

Page 38, first complete paragraph (beginning on line 7)

For 5'-RACE experiments, the Marathon cDNA Amplification kit (Clontech) was used according the manufacturer's instructions with minor modifications. The 5'-untranslated end of the normal PLAG! Transcript as well as the chimeric transcripts were isolated by 5'-RACE. First strand placenta or adenoma cDNA respectively, was synthesized from 5 μg total RNA using the MV2 primer (5'-CTG CAC TTG ACC CAC CCC TGG GAT-3') (SEQ ID NO: 99) located in exon 5. The ds cDNA was ligated to the adaptor and amplified using the anchor primer AP1 and the MV5 primer (5'-CAG GAG AAT GAG TAG CCA TGT GC-3' (SEQ ID NO: 100) also located in exon 5. A second round of PCR was performed using the anchor primer and the MV6 primer (5'-TGC ACT TGT AGG GCC TCT CTC CTG-3') (SEQ ID NO: 101) located in exon 4. The final PCR products were purified out of agarose gel and cloned into th epCRII vector (Invitrogen).

Page 38, the paragraph beginning on line 24 and continuing onto page 39

Total RNA (5 μg) was reverse-transcribed using Superscript II reverse transcriptase (GGIBCO BRL) and oligo d(T) primers according to the recommended conditions. 0.25 μg of the resulting cDNA was subject to amplification using a variety of primer sets. The amplifiation conditions for the CTNNB1/PLAG1 fusion transcripts were 30 cycles at 94 °C for 10 sec and 68 C for 1 min in a final volume of 50 μl using the Expand

long template PCR system (Boehringer Mannheim). The first round PCR was carried out with the CTNNB1 primer 5'-TGT GGC AGC AGC GTT GGC CCG-3' (SEQ ID NO: 102) (CAT-UP) and the PLAG1 primer 5'-CAG GAG AAT GAG TAG CCA TGT GC-3' (MV5). The second round was performed on a 20 fold diluted sample with the CTNNB1 primer 5'-ACG GAG GAA GGT CTG AGG AGC AG-3' (SEQ ID NO: 103) (NECAT-UP) and the PLAG1 primer 5'-TGC ACT TGT AGG GCC TCT CTC CTG-3' (SEQ ID NO: 104) (MV6). To amplify the reciprocal PLAG1/CTNNB1 fusion transcript two rounds of PCR amplification were performed with 30 cycles at 94 °C for 30 sec, 63 °C for 30 sec and 72 °C for 1 min in a final volume of 50 μl. The first round was carried out with the PLAG1 primer 5'-CAA TGG CTG CTG GAA AGA GG-3' (SEQ ID NO: 105) (START-UP) and the CTNNB1 primer 5'-AAG GAG CTG TGG TAG TGG CAC-3' (SEQ ID NO: 106) (CAT3). The second round was performed on a 20 fold diluted sample with the PLAG1 primer 5'-GGC CGG AGG GAG GAT GTT AA-3' (SEQ ID NO: 107) (START RACE) and the CTNNB1 primer 5'-GCC GCT TTT CTG TCT GGT TCC A-3' (SEQ ID NO: 108) (CAT3NEST).

Page 40, second complete paragraph (beginning on line 11)

In initial Northern blot experiments using a PCR probe corresponding to this particular YAC end, a 7.5 kb transcript was detected. A combination of sequential screenings of a human fetal kidney cDNA library as well as 5'- and 3'-RACE experiments led to the isolation of a composite cDNA of 7313 nucleotides (GenBank accession number U65002). This cDNA contains an open reading frame (ORF) of 1500 bp starting with the ATG at position 481-483 (Fig. 4A). An in frame stop condon (TAG) is present 9 nucleotides upstream of this ATG. The deduced amino acid sequence reveals seven canonical C2H2 zinc finger domains (Fig. 4B) and a non-finger region of 259 amino acid residues representing the carboxy-0terminus of the deduced protein. The zinc finger motifs including the linker

sequences are between 28 and 35 amino acids long. The cysteine (C) and histidine (H) residues are present in their characteristic positions in each finger. The typical phenylalanine (F) and leucine (L) residues in finger 4 and 6 are lacking. Strictly spoken, the deduced protein is not a Kruppel zinc finger protein, since it does not contain the characteristic H/C linker (consensus sequence TGEKPYK) (SEQ ID NO: 109) in between the zinc fingers (Bellefroid et al. (1989)). Only the seven amino acids between finger 1 and 2 resemble the H/C linker (TGERPYK) (SEQ ID NO: 110). The amino-terminal region contains two nuclear localization signals (KRKR (SEQ ID NO: 111) and KPRK (SEQ ID NO: 112)). The carboxy-terminus is serine-rich (45 amino acid residues out of 259, i.e. 17%, raising the possibility of a regulatory role that may be controlled by serine/threonine kinases.

Page 56, first complete paragraph (beginning on line 20)

Fine needle biopsies of patients having a pleomorphic adenoma of the salivary gland (with a chromosome 8q12 abberation others with a normal karyotype) were taken. From the material thus obtained total RNA was extracted using the standard TRIZOL™ LS protocol from GIBCO BRL as described in the manual of the maufacturer. This total RNA was used to prepare the first strand of cDNA using reverse transcriptase (GIBCO/BRL) and an oligo dT(17) primer containing an attached short additional nucleotide stretch. The sequence 2.6 [AAG GAT CCG TCG ACA TC (T) 17] (SEQ ID NO: 93). Rnase H was subsequently used to remove the RNA from the synthesized DNA/RNA hybrid molecule. PCR was performed using a gene-specific primer (Example 2, point 2.6) and a primer complementary to the attached short additional nucleotide stretch. The thus obtained PCR product was analysed by gel electrophoresis. Fusion constructs were detected by comparing them with the background bands of normal cells of the same individual.

Page 57, first complete paragraph (beginning on line 1)

In an additional experiment, a second round of hemi-nested PCR was performed using one internal primer and the primer complementary to the short nucleotide stretch [AAG GAT CCG TCG ACA TC(T) 17] (SEQ ID NO: 93). The sensitivity of the test was thus significantly improved.

Page 63, third complete paragraph (beginning on line 26)

For the preparation of rabbit polyclonal antibodies directed against the PLAG1-encoded proteins, use was made of the following three commercailly available peptides:

(H-DLSEVRDTQKVPSGKR) (SEQ ID NO: 113) 8-Multiple Antigen Peptides

(H-FSSTSYAISIPEKEQPL) (SEQ ID NO: 114) 8-MAP

(H-QLPTQTQDLQDP) (SEQ ID NO: 115) 8-MAP

obtainable from Research Genetics Inc. Huntsville, AL, USA. The polyclonal antibodies were made according to standard techniques.

MARKED-UP AMENDED CLAIMS

28. (Twice Amended) The nucleic acid as claimed in claim 47, having homology with the zinc finger domains of the PLAG1 (pleomorphic adenoma gene 1) gene the nucleotide sequence of which is depicted in figure 4A (SEQ ID NO: 116), or a complementary strand thereof, including modified, degenerate or elongated versions of both strands.

29. (Twice Amended) The nucleic acid as claimed in claim 47, comprising the nucleotide sequence of the PLAG1 gene as depicted in figure 4A (SEQ ID NO: 116), or a complementary strand thereof, including modified, degenerate or elongated versions of both strands.